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CLONING OF THE CALCITONIN AND THE CALCITONIN GENE-RELATED PEPTIDE RECEPTOR cDNA AND THE PUTATIVE PHYSIOLOGICAL ROLE OF THESE HORMONES IN FLOUNDER

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In mammals, a single calcitonin (CT) gene generates mRNAs encoding, either CT in thyroidal C-cells or calcitonin gene-related peptide (CGRP) in neural tissues by tissue-specific alternative splicing. CT has a hypocalcemic activity, and CGRP acts as a neuropeptide hormone. In fish, however, little information is available on the functions of CT and CGRP. So, we intended to clone CT receptor (CTR) and CGRP receptor (CGRPR) cDNAs from flounder (*Paralichthys olivaceus*), and to reveal their tissue expression pattern. Both CTR and CGRPR could be cloned from gill cDNA. Deduced CTR amino acid sequence shares 49% identity with human CTR, while CGRPR has a high degree of identity to human CGRPR (72%). In the tissue expression, it was noticeable that both genes are expressed in the gill, heart, intestine, brain, ovary and testis, and that CTR expression is high in heart and low in liver. To estimate the function of two hormones during the adaptation to low salinity conditions, flounder were successively transferred from seawater (SW) to 1/2 SW, 1/4 SW and freshwater, and expression of two receptor mRNAs in the gills was assayed by the RT-PCR. CTR expression in gills did not change during the adaptation, while CGRR expression exhibited strong down-regulation in response to the decrease of salinity. These results are the first step to elucidate the physiological roles of CT and CGRP in fish.

Electrostatic interactions of progesterone and androgen metabolites with rainbow trout estrogen receptor

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In rainbow trout liver, vitellogenin (Vg) synthesis is regulated by estradiol (E_2) acting through the estrogen receptor (ER). However, I found that in primary cultures of immature male rainbow trout hepatocytes, besides E_2, several progesterone and androgen metabolites could stimulate Vg gene expression. Some of these steroids showed minimal conversion into E_2 during 24-hrs incubation with culture medium by ELISA. Moreover, tamoxifen completely inhibited the Vg gene expression they induced, suggesting a possible involvement of rainbow trout ER (rtER) in their stimulatory action. The steroids stimulating Vg gene expression had virtually no, or a slightly positive, charge on the Connolly surface. On the contrary, those that failed to stimulate Vg gene expression had a strong positive or negative charge around rings C and D due to polarization. The amino acid sequence of the ligand binding domain (LBD) of rtER has 57.7% homology with human (h) ERαLBD. Only one amino acid of the direct binding site differs between the two species.

CLONING OF CDNA ENCODING ENDOTHELIN RECEPTOR OF MEDAKA FISH, *ORYZIAS LATIPES*.

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Endothelins are known to participate in physiological processes including vasoconstriction, mitogenesis and embryonic development in vertebrates. Endothelin receptors (EDNRs) that accept the signals of endothelins have been isolated as subtypes of EDNR-A, -B, -B2 and -C from mammalian, avian and amphibian. However, there was no molecular information for EDNR of teleost. In this report, we tried to isolate a cDNA of the EDNR gene of Medaka fish, *Oryzias latipes*. RNA was prepared from adult Medaka fish and RT-PCR was performed with the degenerate primers for the consensus region of the reported EDNR gene. The PCR product was a 300 bp single DNA fragment carrying the expected size. To determine full length of cDNA sequence, we performed 5'- and 3'-RACE using several primers for the isolated DNA fragment, and obtained both franking regions from same RNA. Combining these DNA fragments, complete nucleotide sequence contained 1239 bp ORF region was determined. The deduced amino acid sequence revealed 52%, 60%, 61%, 63% and 50% homology to human EDNR-A, -B, quail EDNR-B, -B2, and *Xenopus* EDNR-C, respectively. The obtained clone seems to be a cDNA encoding EDNR of Medaka fish. Analysis of its expression patterns during Medaka fish development by RT-PCR and southern hybridization is in progress.

EXPRESSION OF TRANSFORMING GROWTH FACTOR-α IN THE MOUSE PITUITARY.

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Transforming growth factor alpha (TGF-α), a member of epidermal growth factor family, is a regulatory peptide produced by a variety of normal and neoplastic tissues. Evidences have shown the expression of TGF-α in the bovine, rat and human pituitaries. This experiment was designed to search the mRNA expression of TGF-α in the mouse pituitary by *in situ* hybridization using paraformaldehyde fixed paraffin sections. TGF-α was detected in the anterior lobes of the pituitary gland of adult male and female ICR mice. About 65% of the cells expressed TGF-α in males whereas only about 50-55% were expressed in females. Further, TGF-α was detected in the somatotrophs, but was not detected in the mammotrophs. Our recent study showed that TGF-α stimulates poliferation of mammotrophs in the mouse pituitary. TGF-α produced in somatotrophs stimulates the growth of mammotrops in a paracrine manner.

EXPRESSION OF GROWTH HORMONE RECEPTOR IN THE MOUSE PITUITARY GLAND

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Growth hormone (GH) is synthesized in pituitary glands. GH exerts its actions directly on various target organs.GH plays some roles in the pituitary glands. However, molecular mechanism of their GH actions within the pituitary gland was poorly understood. The present study was designed to examine GH-receptor (GHR) expression in the ICR mouse pituitary glands. Detection of GHRs was carried out by *in situ* hybridization using mouse GHR cDNA probe. GHR mRNA was detected in the anterior lobes , but not in the intermediate and neural lobes. Secretory cell types of GHR-expressing cells were immunocytochemically determined using antisera against pituitary hormones. GHR mRNA was detected in somatotrophs. This result suggests that GH regulates somatotrophic function through GHRs in an autocrine manner.

ESTABLISHMENT AND ANALYSIS OF MOUSE OVIDUCTAL CELL LINES

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The mammalian oviduct provides an environment for the survival and transport of gametes, fertilization and development of early embryos. Studies of oviductal functions have been conducted mainly in primary cell and organ cultures. However, the small size of the oviduct limits the sources of both epithelium and stroma. To circumvent the limit, we tried to establish cell lines from oviducts of p53-deficient mice, which were shown to provide clonal cell lines. An oviduct was divided into three parts: infundibulum, ampulla, and isthmus, and cultured after enzymatic digestion. They were maintained in medium containing 10% fetal calf serum supplemented estradiol-17β at 10^{-8} M. More than 200 clonal lines were established from every region. Morphologically identical lines were frozen and 32 lines were selected. They express cytokeratin or vimentin and receptors for estrogen and progesterone. Growth effects of sex steroids and growth factors were examined on these lines.

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